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The past three years of work for the Air Force Office of Scientific Research has resulted in the development of a congenic mouse model of JP-8 jet fuel exposre, the role of substance P in the JP-8 jet fuel-induced lung injury process, and development of extensive collaborations with Dr David Harris (University of Arizona), Drs Kornguth and Siegel (University of Wisconsin), and Dr Frank Witzman (Indiana University). We demonstrated that congenic mice deficient in the aryl hydrocarbon hydroxylase and N-acetyl transferase enzymes have increased lung permeability and pathological lung injury resulting from exposure to JP-8 jet fuel compared to their C57BL/6 parent strain. Consequently, we can conclude that one or both of these enzymes plays a role in the metabolism of JP-8 fuel in the lungs. Finally, we have conducted field studies for JP-8 jet fuel exposure at the Montana Air National Guard Base in Great Falls, Montana in March of 1997 and at Davis Monthan Air Force Base in Tucson, Arizona. The purpose of this semi-cold weather (30 degree) F-16A engine start and warm weather (102 degree) F-16A engine start were to determine "real-life" JP-8 jet fuel exposures at teh ground crew positions and determine average JP-8 jet fuel concentration and particle size. The data was then compared against similar data generated in our jP-8 jet fuel exposre model.

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SUMMARY ABSTRACT

The past three years of work for the Air Force Office of Scientific Research has resulted in the development of a congenic mouse model of JP-8 jet fuel exposure, the role of substance P in the JP-8 jet fuel-induced lung injury process, and development of extensive collaborations with Dr. David Harris (University of Arizona), Drs. Kornguth and Siegel (University of Wisconsin), and Dr. Frank Witzman (Indiana University).

We demonstrated that congenic mice deficient in the aryl hydrocarbon hydroxylase and N-acetyl transferase enzymes have increased lung permeability and pathological lung injury resulting from exposure to JP-8 jet fuel compared to their C57BL/6 parent strain. Consequently, we can conclude that one or both of these enzymes plays a role in the metabolism of JP-8 jet fuel in the lungs.

In the past year of work, we have determined a possible role for substance P in the clearance of JP-8 jet fuel from the lungs. The work conducted by my doctoral student, Raymond F. Robledo, was centered about the development of a gas chromatography-mass spectroscopy method for determination of various JP-8 jet fuel metabolites in lung tissue. Preliminary results indicate that [Sar^9, Met $(O_2)^{11}$]-substance P increases JP-8 jet fuel clearance by approximately 50% within 15 minutes after an acute jet fuel exposure. Mr. Robledo will present this work at the International Tachykinins Conference in Cairns, Australia in September 1997.

Additional work in the last two years centered about acute exposure to JP-8 jet fuel of between 1000 mg/m^3 and 2500 mg/m^3. This insult caused pulmonary injury that was characterized by increased alveolar permeability to ^{99m}Tc-DTPA and bronchoalveolar lavage fluid (BALF) increases of total protein, lactate dehydrogenase (LDH), N-acetyl-ß-D-glucosaminidase (NAG), and alveolar macrophages. Seven day sub-chronic exposures have resulted in a threshold response at 1000 mg/m^3, with increased ^{99m}Tc-DTPA permeability and increases in BALF total protein and LDH. Sub-chronic exposed mice also had type II alveolar epithelial cell alterations and necrotic Clara cells that may have contributed to the observed increase in total pulmonary compliance. In contrast to the acute exposures, sub-chronic exposures had a decrease in BALF alveolar macrophages and NAG. Using a congenic mouse strain (aryl hydrocarbon hydroxylase and N-acetyltransferase enzyme deficient) that has been shown to be sensitive to JP-8 jet fuel, the objective of this research was to determine if [Sar^9, Met $(O_2)^{11}$]-substance P (substance P receptor agonist) administration could attenuate pulmonary toxicity to JP-8 jet fuel. Mice administered [Sar^9, Met $(O_2)^{11}$]-substance P (1 µM aerosol), for 15 min immediately following each 1000 mg/m^3 JP-8 jet fuel exposure, had control values for all pulmonary injury biomarkers. To further support these findings, mice were treated with CP-96,345 (substance P receptor antagonist, 2.5 mg/kg, i.p.) prior to each JP-8 jet fuel exposure. CP-96,345 pretreated mice had a potentiated increase in ^{99m}Tc-DTPA permeability and a potentiated decrease in alveolar macrophages compared to mice exposed to 1000 mg/m^3 JP-8 jet fuel alone. These mice also had an increase in in type II alveolar epithelial cell alterations, Clara cell necrosis, and pulmonary edema.

Finally, we have conducted field studies for JP-8 jet fuel exposure at the Montana Air National Guard Base in Great Falls, Montana in March of 1997 and at Davis-Monthan Air Force Base in Tucson, Arizona. The purpose of this semi-cold weather (30° F) F-16A engine start and warm weather (102° F) F-16A engine start were to determine "real-life" JP-8 jet fuel exposures at the ground crew positions and determine average JP-8 jet fuel concentration and particle size. This data was then compared against similar data generated in our JP-8 jet fuel exposure model. We determined that our mean aerosol

particle size of 1.2 μm was very comparable to the particle size range determined in our F-16A engine start field tests which ranged from 0.7 to 1.5 μm.

PUBLICATIONS FROM PROJECT: To May 14, 1997

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PARTICIPATING PROFESSIONALS

(1)	Mark L. Witten, Ph.D. University of Arizona College of Medicine	Principal Investigator
(2)	Raymond F. Robledo University of Arizona College of Pharmacy	Doctoral Student
(3)	Veronica Breceda University of Arizona	Research Associate
(4)	Susan E. Leeman, Ph.D. Boston University College of Medicine	Consultant
(5)	Robert C. Lantz, Ph.D. University of Arizona College of Medicine	Co-Investigator
(6)	Dr. David T. Harris, Ph.D. University of Arizona College of Medicine	Co-Investigator

(7) Carol M. Baldwin, Ph.D. — Post-Doctoral Fellow
University of Arizona College of Medicine

(8) R. Scott Young — Research Technician
University of Arizona College of Medicine

(9) Dr. Carol Barnes — Neuroscience Consultant
University of Arizona College of Medicine

COUPLING ACTIVITIES

As stated previously in this report, we have active collaborations with the following investigators:

(1) Dr. David T. Harris — University of Arizona
(2) Drs. Siegel & Kornguth — University of Wisconsin
(3) Dr. Frank Witzmann — Indiana University

We are coordinating our JP-8 jet fuel research with the following Air Force agencies:

(1) Major Wade H. Weisman
Toxicology Division, Armstrong Laboratory, Wright-Patterson AFB, Ohio
(2) Captain Les Miller
Toxicology Division, Armstrong Laboratory, Wright-Patterson AFB, Ohio
(3) Major Leslie B. Smith, Head, JP-8 IPT.
Industrial Hygiene Consultant, Armstrong Laboratory, Brooks AFB, Texas
(4) Major Gary Carlton
Chief, Industrial Hygiene Branch, Armstrong Laboratory, Brooks AFB, Texas

DISCOVERIES, INVENTIONS, PATENT DISCLOSURES, AND SPECIFIC APPLICATIONS

A provisional patent application entitled, "Substance P for Treatment of Immunosuppression" was filed with the United States Patent Office in Crystal City, Virginia on July 23, 1996. This provisional patent application originated from work performed in our Air Force Office of Scientific Research-sponsored JP-8 jet fuel research in conjunction with Dr. David Harris who is also supported by the Air Force Office of Scientific Research. Because of the laws and regulations involving "public disclosure" in the patent process, we have been unable to generate manuscripts for publication until the regular patent application was filed on March 28, 1997. We will file for world-wide patent application by July 23, 1997. We are in the process of commercializing this patent application and at this point in time have negotiations underway with three pharmaceutical companies and one investment capital firm.

RESEARCH ACCOMPLISHMENTS

In the past three years, we have established that JP-8 jet fuel metabolism in the lungs is dependent upon either or both the aryl hydrocarbon hydroxylase enzyme and N-acetyl transferase enzyme. Additionally, we have determined that substance P administration attenuates JP-8 jet fuel-induced lung injury, possibly by "clearing" the jet fuel from the lungs in an expeditious manner. Finally, we have determined the extent of lung injury from JP-8 jet fuel exposures in the range of 1,000 to 2,500 mg/m^3.

We believe that our patent entitled "Substance P for Treatment of Immunosuppression" may have clinical benefits in the treatment of AIDS, maintaining the immune systems of both elderly and young patients, as a vaccine additive to boost the immune system's response to the vaccine, possible therapy for environmental toxicants such as cigarette smoke exposure and air pollution, and bone marrow transplants.

We have also correlated our JP-8 jet fuel exposure model with "real-life" jet fuel exposure situations in regular Air Force operations in a semi-cold weather F-16A engine start at the Montana Air National Guard Base and a warm weather F-16A engine start at Davis-Monthan AFB, Arizona.

Finally, Mr. Robledo's abstract at the 1997 Society of Toxicology National Meeting in Cincinnati, Ohio was awarded the "Outstanding Graduate Student Presentation Award" in the inhalation toxicology section in March of 1997.